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Poly(3-hydroxybutyrate): Promising biomaterial for bone tissue engineering

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Poly(3-hydroxybutyrate) is a natural polymer, produced by different bacteria, with good biocompatibility and biodegradability. Cardiovascular patches, scaffolds in tissue engineering and drug carriers are some of the possible biomedical applications of poly(3-hydroxybutyrate). In the past decade, many researchers examined the different physicochemical modifications of poly(3-hydroxybutyrate) in order to improve its properties for use in the field of bone tissue engineering. Poly(3-hydroxybutyrate) composites with hydroxyapatite and bioglass are intensively tested with animal and human osteoblasts *in vitro* to provide information about their biocompatibility, biodegradability and osteoinductivity. Good bone regeneration was proven when poly(3-hydroxybutyrate) patches were implanted *in vivo* in bone tissue of cats, minipigs and rats. This review summarizes the recent reports of *in vitro* and *in vivo* studies of pure poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate) composites with the emphasis on their bioactivity and biocompatibility with bone cells.

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INTRODUCTION

A biomaterial can be defined as a natural or synthetic material suitable for interacting with biological systems, with a function to treat, augment or replace any tissue or organ (1). Biodegradable and biocompatible natural or synthetic polymers are referred to as "biomaterials" (2). Biomaterials for bone tissue engineering should fulfill the following requirements (3–5):

- Mechanical strength to withstand hydrostatic pressure. Young's modulus of cortical bone is between 15 and 20 GPa and that of cancellous bone is between 0.1 and 2 GPa. Compressive strength varies between 100 and 200 MPa for cortical bone, and between 2 and 20 MPa for cancellous bone (5). The large variation in mechanical properties and geometry makes it difficult to design an "ideal" bone scaffold.

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- Biocompatibility to induce new tissue formation without inflammation.
- Surface properties that suit osteogenic cells. For growth, proliferation and differentiation of cell hierarchical structure is crucial.
- Osteoinductivity to promote migration of osteogenic cells and to stimulate differentiation. An important role in osteoinductivity is played by chemical composition of the scaffold, porosity, surface properties and nano/micro topography.
- Porosity to allow cell ingrowth and neovascularization. Porosity is essential for diffusion of nutrients and for removal of metabolic wastes resulting from cellular activity. Pore size should be at least 100 μm in diameter for successful diffusion of essential nutrients and oxygen. However, pore size in the range of 200 to 350 μm was found to be optimal for bone tissue in-growth (4, 5).
- Vascularity to stimulate angiogenesis. Lack of vasculature leads to ischemia and cell apoptosis.
- Bioresorbability after scaffold degradation to allow new bone tissue to grow. The scaffold should degrade at a controlled resorption rate, creating space for the new bone tissue to grow. Degradation products should not cause inflammation to the surrounding tissue (3–5).

Biodegradable and biocompatible polyesters are being investigated worldwide for pharmacological, biomedical and environmental purposes (6). Polyhydroxyalkanoates (PHA) are natural polyesters produced by bacteria as intracellular carbon and energy sources when essential nutrients are limited and carbon is available (7). Poly(3-hydroxybutyrate) (PHB) is one of the best-known polymers of the PHA family (8). PHB is a homopolymer of (*R*)-3-hydroxybutyrate units (Fig. 1). Its molecular mass can range from 200 to up to 20,000 (9, 10).

Molecular mass of PHB is a very important feature to consider in PHB applications, because it determines the mechanical properties of the polymer and, in turn, the final application. Mechanical and thermal properties of PHB are high crystallinity (60–70 %), high melting temperature (175 °C), good tensile strength (30–35 MPa) and appropriate elasticity modulus (3 GPa) (11, 12). It is a promising material for biomedical applications because it is a natural, renewable, biodegradable and biocompatible thermoplastic (13). PHB decomposes to 3-hydroxybutyric acid, which is also normally found in human blood (14). 3-Hydroxybutyric acid increases calcium influx in cultured cells and suppresses their death (15). In nature, PHB is degraded by the action of non-specific lipases and esterases. Therefore, lipases and esterases are presumably the enzymes that degrade PHB implants and their medical devices *in vivo* (16). Furthermore, sterilization of PHB-based materials does not affect the mechanical or chemical properties (17). Nevertheless, for packing materials, tissue engineering, and other specific applications, the physical and mechanical properties

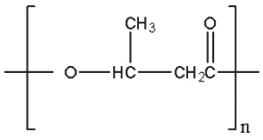

Fig. 1. Chemical structure of poly(3-hydroxybutyrate) (PHB).

of PHB need to be diversified and improved. The main limitation, besides its relatively high cost of production, is the lack of bioactivity (12, 16). In addition, hydrophobic PHB needs to have a hydrophilic character for biomedical applications (2). Porosity, surface properties and, in part, mechanical strength are determined by processing conditions when preparing a scaffold for bone tissue engineering (4). PHB, like all PHAs, is sensitive to processing conditions, especially to temperature and shear and exhibits a very narrow processing window (12). For example, PHBs of low molecular mass ($< 1 \times 10^3$) are characterized by early thermal degradation, near their melting temperature (above 180 °C) (12, 18). Additives, blends and composites are mostly used to overcome these problems (8). Furthermore, PHB is soluble in few solvents, *i.e.*, chloroform, dichloromethane and dimethyl formamide; therefore, the formation of composite structures is challenging (19). As already mentioned, thermal molding is also difficult, since above 150 °C most of the PHA-based polymers break down to fatally toxic *trans*-crotonic acids. Taken together, PHB limitations such as mechanical properties, high production cost, limited functionalities, incompatibility with conventional thermal processing techniques, susceptibility to thermal degradation have limited effective application of PHB and these are still challenges to be addressed in the future. In the present review, we report on the current biomedical applications of PHB in bone tissue engineering with the emphasis on physicochemical modifications of PHB and *in vitro* and *in vivo* experiments.

BIOMEDICAL APPLICATIONS OF PHB IN BONE TISSUE ENGINEERING

Physicochemical modifications of PHB in the field of bone tissue engineering

Numerous studies investigate the use of PHB in bone tissue engineering (20). Bone tissue engineering is a research area where bone replacements are being developed and clinically tested in cases of orthopedic defects, bone tumors, and in maxillofacial, neck and head surgery (11). It provides solutions for generating new bone tissue with good functional and mechanical qualities (21). Recent work in this field has been focused on the development of three-dimensional porous scaffolds loaded with specific living cells to provide tissue regeneration in a natural way. According to Hutmacher (22), a scaffold should satisfy the following criteria: (*i*) to be bioresorbable and biocompatible with a controllable degradation and resorption rate to match cell/tissue growth *in vitro*/*vivo*; (*ii*) to have suitable surface chemistry for cell attachment, proliferation and differentiation; (*iii*) to be three-dimensional and highly porous to enable cell growth, flow transport of nutrients and metabolic waste; (*iv*) to have proper mechanical properties like the tissue at the site of implantation. In addition to being biocompatible and biodegradable, PHB-based biomaterials are piezoelectric and thus may promote bone growth *in vivo* (23). PHB has the disadvantages of having low compressive modulus and poor bioactivity. Ceramic implants, on the other side, are stiffer but they are often fragile and known to fracture during clinical use (24). Therefore, a combination of PHB with a bioactive ceramic is expected to improve the mechanical and chemical properties of composites (24).

Bone is considered to be a composite material consisting of nanosized calcium phosphates (CaP) embedded in a collagen-rich organic matrix permeated with pores filled with liquids (24). Most similar to the mineral part of the bone is hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA] (24). Its chemical similarity to the inorganic bone materials makes it biocompatible,

modifiable by the osteoclasts and slowly biodegrading *in situ* (25, 26). As HA is a porous material, ingrowth of capillaries and other vessels is possible (27). In this way, the cells in the scaffold are supplied with metabolic oxygen and nutrients. Wang *et al*. (28) showed that growth and alkaline phosphatase activity of osteoblasts were better on PHB-HA scaffolds compared to PHB scaffolds. According to Hayati *et al*. (11), 15 % (*m*/*m*) of HA nanoparticles was the best content for incorporation of HA in a PHB matrix, while Shishatskaya *et al*. (29) obtained the best results for growth and differentiation of osteoblasts on PHB/HA composites containing 10 and 20 % HA. Sadat-Shojai *et al*. (24) have proven that 15 % (*m*/*m*) of HA nanoparticles stimulate cell proliferation and cell differentiation. HA nanoparticles covering the PHB fiber surface enhanced differentiation of mesenchymal stromal cells toward the osteoblast phenotype (30–32). Titanium oxide (TiO_2) showed no effect on osteoblast growth when TiO_2 was added to PHB/HA scaffolds (34). The above mentioned studies also reported improved mechanical properties of PHB/HA scaffolds (3, 11, 24, 32). Bioglass with osteoconduction and osteostimulation properties is another bioactive inorganic phase used in making composites with PHB. It has been shown that optimal concentration of nanobioglass (7.5 %, *m*/*m*) in nanocomposite scaffolds significantly improves cell proliferation and induces better cytocompatibility and osteoconductivity compared to PHB scaffolds (35). Bioactivity of PHB composites with bioglass (10 %, *m*/*m*) was also proven by Misra *et al*. (36). Nanobioglass was shown to improve mechanical strength and increase the scaffold degradation kinetics (35, 36). The latest study evaluated the response of bone to novel biodegradable polymeric composite implants made of PHB and Herafill® (37). Herafill® is a composite made of calcium sulfate ($CaSO_4$), calcium carbonate ($CaCO_3$) and glycerol tripalmitate (37). It was clinically used as an alternative bone substitute material with proven osteoconductivity (38). The highest value of bone accumulation was observed around the implant of the PHB composite with 30 % of Herafill®; however, the authors could not give any clear recommendation regarding the use of PHB composite materials as biodegradable implants for bone fixation (37). As a natural polysaccharide, chitosan is known for its biocompatibility and therefore PHB/HA/chitosan composite scaffolds (39) and PHB/ biophasic calcium phosphate/chitosan membranes (40, 41) were tested for their use in bone tissue engineering. Chitosan reduced crystallinity and improved surface properties and biological activity of scaffolds (3, 20, 39). Most recent studies report modifications of PHB with poly(ε-caprolactone) (PCL) and sol-gel silica (42), PCL and bioglass (43), cellulose acetate (44), zwitterionic poly(4-vinylpiridine) hydrophilic groups on poly(octadecylacrylate) blocks (45) and natural anionic polysaccharides (46). Sol-gel silica enhanced the stiffness and strength of PHB/PCL fibers (42), while PHB/cellulose acetate scaffolds had three times higher degradation rates compared to PHB scaffolds (44). Incorporation of natural anionic polysaccharides into PHB decreased its crystallinity, enhanced surface hydrophilicity, reduced brittleness and enhanced degradation of polymer blend films (46). All five studies have proven cell growth and proliferation on those scaffolds; nevertheless, further research is needed. Physicochemical modifications of PHB are presented in Table I.

In vitro *biocompatibility of PHB on bone cells*

In vitro tests of PHB and PHB composite scaffolds were performed with osteoblasts of different organisms (16). Polymeric materials, bone cell types and methods of analysis of biocompatibility tests *in vitro* are reported in Table II. Wang *et al*. (28, 48) evaluated the

Table I. Physicochemical modifications of PHB in the field of bone tissue engineering

Modifier	Concentration in PHB (%, *m*/*m*)	Porosity (%)	Enhanced properties	Reference
HA	10	Solid film	Bioactivity	28
	10–20	Solid film		29
	15	77		11
	15	Solid film		24
	20	71(blend)/83(spray)		30
	10	84		31
	5	82		32
	15	Not reported		33
HA/TiO_2	50	76	Cell binding	34
Herafill®	30	Solid film	Bioactivity	37
Bioglass	7.5	80	Bioactivity	35
	10	85	Bioactivity	36
CP/chitosan	3/10	41	Bioactivity	40
CP/chitosan	50	Not reported	Bioactivity	41
PCL/silica	Not reported	Not reported	Bioactivity	42
PCL/bioglass	5	Not reported	Bioactivity	43
CA	10, 20, 30, 40	86–81	Cytocompatibility	44
4VP-*r*-ODA	Not reported	36	Bioactivity	45
Natural anionic polysaccharides	5, 10, 20, 30, 50	Not reported	Bioactivity	46
Magnesium discs with PHB coating	–	Not reported	Bioactivity	47

CA – cellulose acetate, CP – calcium phosphate, PCL – poly(ε-caprolactone), 4VP-*r*-ODA – zwitterionic poly(4-vinylpiridine) hydrophilic groups on poly(octadecylacrylate) blocks

attachment, proliferation and differentiation of rabbit bone marrow cells on PHB and PHB-HA scaffolds. PHB-HA scaffolds were shown to be a suitable biomaterial for rabbit bone marrow cell attachment, proliferation and differentiation (28, 32, 48). Several studies established *in vitro* tests of PHB scaffolds on murine osteoblasts (6, 7, 29). Sadat-Shojai *et al*. (24) showed a significant increase in proliferation and differentiation of murine osteoblasts on PHB/HA composites. A novel study by Zhijiang *et al*. (44) demonstrated that the PHB/cellulose acetate blend nanofiber scaffolds have better biocompatibility and higher proliferation rate of murine osteoblasts than a pure PHB film. In their recent studies, Sadat-Shojai *et al*. describe a new strategy for fabrication of bone scaffolds using electrospun nano-HA/PHB fibers (33) and electrospun nano-HA/PHB and protein gels (49). According to their results, mechanical properties of the construct were good and murine osteoblasts inside the scaffolds were viable. Since cells rapidly proliferate on PHB scaffolds, Peng *et al*. (50)

Table II. Polymeric materials, bone cell types and methods of analysis of biocompatibility tests in vitro

Polymeric material	Bone cell type	Methods of analysis	Reference
PHB scaffolds	Rabbit bone marrow cells	SEM analysis MTT assay ALP assay	48
PHB/HA scaffolds	Rabbit bone marrow cells	SEM analysis MTT assay ALP assay	28
PHB/HA scaffolds	Rabbit bone marrow cells	SEM analysis MTT assay ALP assay RT-PCR	32
PHB/HA composites	Murine osteoblasts	SEM analysis MTT assay	29
PHB and poly(ε-caprolactone) copolymers	Murine osteoblasts (MC3T3-E1)	SEM analysis MTT assay	6
PHB scaffolds coated with collagen I and chondroitin sulfate	Human mesenchymal stem cells (hMSCs)	SEM analysis ALP assay	52
PBS/bioglass scaffolds	Human osteosarcoma cell line (MG-63)	SEM analysis Alamar blue assay	36
PHB films	Rat osteoblasts	SEM analysis BrdU incorporation Flow cytometry qRT-PCR	50
PHB scaffolds	Mouse mesenchymal stem cells (mMSCs)	SEM analysis MTS assay	7
PHB/bioglass scaffolds	Human osteosarcoma cell line (MG-63)	SEM analysis MTT assay Trypan blue staining ALP assay	35
PHB/HA scaffolds	Human osteosarcoma cell line (MG-63)	SEM analysis MTT assay	11
PHB/HA scaffolds	Human osteosarcoma cell line (MG-63)	SEM analysis MTT assay ALP assay	31

Polymeric material	Bone cell type	Methods of analysis	Reference
PHB/HA scaffolds	Murine osteoblasts (MC3T3-E1)	SEM analysis Calcein-AM/EthD-1 live/dead kit Alamar blue assay DNA quantification Phalloidin assay Alizarin red staining ALP assay	24
PHB/bioactive glass filler	Human bone marrow cells	SEM analysis MTT assay ALP assay Phalloidin assay	23
PHB/HA scaffolds	Human mesenchymal stromal cells (hMSCs)	SEM analysis MTS assay ALP assay Alizarin red staining	30
PHB and biphasic calcium phosphate/chitosan membranes	Rat osteoblasts	MTS assay	40
PHB membranes designed by NaOH based alkaline treatment	Human osteoblasts	SEM analysis MTT assay	54
PHB/PCL/silica scaffolds	Human osteosarcoma cell line (MG-63)	SEM analysis Phalloidin assay CCK-8 assay ALP assay	42
PHB/HA scaffolds	Human adipose-derived stem cells (HADSCs) co-cultured with human osteoblasts (HOB)	SEM analysis Alamar blue assay ALP assay Alizarin red staining qRT-PCR	55
PHB/HA/TiO_2 scaffolds	Human osteoblast cell line	SEM analysis MTT assay	34
PHB/cellulose acetate scaffolds	Murine fibroblasts (3T3)	SEM analysis MTT assay	44
Magnesium disks with PHB coating	Murine osteoblasts (MC3T3-E1)	SEM analysis MTT assay	47

Polymeric material	Bone cell type	Methods of analysis	Reference
PHB/chitosan/ calcium phosphate films	Murine osteoblasts (MC3T3-E1)	SEM analysis MTS assay ALP assay	41
PHB/HA nanohybrids	Murine osteoblasts (MC3T3-E1)	SEM analysis Calcein-AM/EthD-1 live/dead kit ALP assay Alamar blue assay Phalloidin assay DAPI staining	33
PHB/HA scaffolds with protein hydrogel	Murine osteoblasts (MC3T3-E1)	SEM analysis Calcein-AM/EthD-1 live/dead kit Alamar blue assay DNA quantification Phalloidin assay ALP assay	49
PHB/PCL/bioglass scaffolds	Human osteosarcoma cell line (MG-63)	SEM analysis CCK-8 assay ALP assay Alizarin red staining	43
PHB surface treated by KrF laser	Human osteosarcoma (U-2 OS)	SEM analysis Phalloidin assay DAPI staining	56

Alamar blue assay – quantifying the metabolic activity; Alizarin red staining – *in vitro* calcium containing deposits and mineralized matrix analysis; ALP assay – alkaline phosphatase activity assay; BrdU incorporation – staining of 5-bromodeoxyuridine, evaluation of cell proliferation; Calcein-AM/EthD-1 live/dead kit – calcein-AM/ethidium homodimer-1, visualization of cell viability; CCK-8 – cell counting kit-8; DAPI – 4′,6-diamidino-2-phenylindole; DNA quantification – determination of cell proliferation; ECM – extracellular matrix; ELISA – enzyme-linked immunosorbent assay; MTT assay – 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide, *in vitro* mitochondrial metabolic activity test; MTS assay – 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethophenyl)-2(4-sulfofenyl)-2*H*-tetrazolium, *in vitro* cells viability test; Phalloidin assay – *in vitro* immunofluorescence analysis; qRT-PCR – quantitative real-time-polymerase chain reaction, *in vitro* gene expression test; SEM – scanning electron microscopy; Trypan blue staining – quantification of viable cells

tested the risk of carcinogenicity. Their results confirmed no tumor induction when proliferating rat osteoblasts were grown on PHB films. Another study on fibroblasts has proven that PHB is not genotoxic and did not alter the expression of the proto-oncogenes and anti-apoptotic genes analyzed in the study (51). Wang *et al*. (52) proved proliferation of rat osteoblasts on various PHA films; however, the lowest percentage of apoptotic cells was seen when cells were grown on PHB. Tai *et al*. (40) developed asymmetric membranes of

Table III. Polymeric materials and places of PHB implantations in animal bone

Polymeric material	Place of implantation	Reference
PHB patches	Anterior skull base of minipigs	57
PHB/HA composites	Cat femur	59
PHB composites with zirconium dioxide and crystalline Mg	Rat femur	60
PHB patches	Surgically created defects on male rats' cranium	58
PHB composites with zirconium dioxide and Herafill®	Growing rats' femur	37

PHB and biphasic calcium phosphate/chitosan and showed increased proliferation of rat primary osteoblasts on membranes. When PHB/calcium phosphate/chitosan composite films were enzymatically degraded, significant proliferation of murine osteoblasts was observed (41).

Since PHB is a candidate for use in human bone engineering, numerous studies are aimed at assessing biocompatibility using human osteoblasts. Hayati *et al*. (11) and Saadat *et al*. (31) confirmed the biocompatibility of PHB/HA composite scaffolds using the human osteosarcoma cell line (MG-63). Morphology of the attached MG-63 cells in direct contact with the scaffolds demonstrated appropriate cell-scaffold interaction. Similar results on the appropriate cell-scaffold interaction were obtained when MG-63 cells were grown on PHB composite scaffolds with bioglass nanoparticles (35, 36). MG-63 cells showed good osteoblastic differentiation on both PHB/PCL/sol-gel derived silica hybrid scaffolds (42) as well as on PHB/PCL/bioglass hybrid scaffolds (43). Differentiation of human bone marrow cells toward osteoblasts was studied and proven on novel acrylic bone cement combining PHB and bioactive glass (23). Ramier *et al*. (30) developed different types of PHB-based nanofibrous scaffolds and tested them with human mesenchymal stromal cells (hMSCs). Faster cell development was recorded on gelatin-containing scaffolds, whereas HA nanoparticles covering the scaffold surface enhanced differentiation of hMSCs towards the osteoblast phenotype. Rentsch *et al*. (53) seeded hMSCs on PHB scaffolds coated with extracellular matrix components type I collagen and chondroitin sulfate. Their study demonstrated the positive effect of collagen I and chondroitin sulfate on proliferation and differentiation of hMSCs. Karahaliloğlu *et al*. (54) used a NaOH-based alkaline treatment to create PHB membranes and proved increased proliferation of human osteoblasts on the NaOH-treated PHB membranes. Furthermore, NaOH-treated PHB surfaces inhibited *Staphylococcus aureus* growth compared to the untreated PHB surface. Pourmollaabbassi *et al*. (34) evaluated the growth and adhesion of human osteoblasts on PHB/HA/TiO_2 scaffolds. They observed no effect of TiO_2 on cell growth and, therefore, concluded that HA alone affected the growth and cell osteoblast adhesion on the scaffold. A novel study by Rozila *et al*. (55) evaluated the osteogenic potential of human adipose-derived stem cells (HADSCs) when co-cultured with human osteoblasts (HOBs) on electrospun PHB/HA scaffolds. The highest alkaline phosphatase (ALP) production and calcium deposition were shown in the monoculture of HOBs on PHB/HA scaffolds. Nevertheless, co-culture

of HADSCs/HOBs 1:1 on PHB/HA scaffold showed significantly higher cell proliferation, production of ALP, extracellular mineralization and osteogenic-related gene expression compared to other tested groups. The authors concluded that the good interaction of HADSCs and HOBs enhanced the differentiation of stem cells. However, osteogenesis is promoted not only by cell-cell contacts, but also by the bioactive composition of the PHB/HA-based scaffold (55). In the latest study, the growth of human osteosarcoma cells was determined after PHB surface was treated with a KrF laser (56). The proposed method was shown to be suitable for certain modifications in surface properties of the PHB scaffold.

In vivo *studies of PHB in bone*

The following level in evaluating biocompatibility of polymer scaffolds is to observe the response when they are implanted into tissue (Table III).

Bernd *et al*. (57) used PHB patches to cover anterior skull base defects in minipigs. The results showed increasing closure of bone defect with time. After 9 months, the anterior skull base defect was completely closed. Analysis of biodegradation detected a continuous breakdown of PHB. Gredes *et al*. (58) studied PHB patches after implantation in surgically created defects on the cranium of adult rats. No sign of cellular inflammation or PHB rejection was detected. Twelve weeks after surgery, bone formation was proven in all PHB-treated cavities. Furthermore, a pronounced development of blood vessels was observed. However, the authors suggested that the osteoinductive properties of PHB should be further analyzed (58). On the other hand, Alves *et al*. (59) observed a chronic local inflammatory response when PHB/HA composites were implanted in cats. Brigham and Sinskey (16) summarized early *in vivo* biocompatibility studies of different PHA matrices and showed that post-traumatic inflammation following surgical procedures was common around PHA implants. Celarek *et al*. (60) designed a study to evaluate PHB composites with zirconium dioxide, crystalline magnesium alloys and MgZnCa bulk metallic glasses as possible candidates for bone implants. PHB composites were implanted in rats' femora. According to the authors, mechanical properties and degradation of studied materials were unsatisfactory. Meischel *et al*. (37) evaluated the response of bone to PHB composite implants in the femora of growing rats. PHB composites were made with zirconium dioxide and Herafill®. After 36 weeks *in vivo*, no significant degradation in any of the implants was found. Composites containing Herafill® were the most attractive for bone cells with regard to accumulation and growth of bone cells.

As shown above, the results obtained by the *in vivo* studies demonstrated that the PHB-based systems are promising candidates for bone repair. Unfortunately, only few examples of *in vivo* studies involving PHB and PHB scaffolds are present in the literature (37, 57–60). Therefore, more research is needed to confirm and validate the possibility of using this polymer in biomedicine.

CONCLUSIONS AND OUTLOOK

The field of bone tissue engineering has progressed rapidly over the past years. Use of natural polymers seems promising in the bone regeneration process. PHB is a bacterially derived polymer known to be biocompatible and biodegradable. Due to its poor mechanical properties, PHB needs to be modified in order to be useful in biomedical applica-

tions. Different physicochemical modifications of PHB in the field of bone tissue engineering are proposed to improve the PHB properties. Most commonly used modifiers are HA, bioglass and chitosan in combination with calcium phosphate. All three modifiers improved mechanical properties of tested scaffolds compared to neat PHB. Furthermore, the use of HA, bioglass, cellulose acetate or natural anionic polysaccharides as PHB modifiers decreased the degradation rate of tested scaffolds. Enhanced bioactivity is a common feature of all modifiers reported in this review. On the other hand, mechanical properties of human bones vary depending on the species, race, sex and age. Regional variation in mechanical properties is also observed within the same body. Therefore, scaffolds will need to be fabricated accordingly to match the mechanical properties of each respective application. To our knowledge, no study has compared the mechanical properties, osteoinductivity and biodegradability of PHB composites when implanted in male and female test animals yet.

Biocompatibility evaluations conducted under different experimental conditions and using various cell lines highlighted the good *in vitro* and *in vivo* biocompatibility of PHB and PHB composites. Furthermore, PHB/HA composites were proven to enable differentiation of human stem cells toward osteoblast phenotype. Thus, the conclusion was made that PHB/HA scaffolds promote osteogenesis. Studies proved HA to be the optimal modifier for PHB, since PHB/HA scaffolds were shown to have good mechanical properties, optimal osteoinductivity, suitable porosity and, finally, the desired rate of degradability. *In vivo* studies showed new bone formation around PHB patches but slow degradation of PHB implants. Sufficient vascularization of implants would certainly improve cell growth and enable faster degradation of biomaterial. Nevertheless, vascularization *in vivo* is dependent on scaffold structure, namely, pore size and interconnectivity of pores. Also, local delivery of angiogenic growth factors would accelerate vascularization of an implanted graft. Angiogenic growth factors may also be incorporated into the scaffold. The major limitation of *in vivo* results is the small number of such studies; therefore, further research is needed. To our knowledge, PHB has not received approval for uses in biomedicine either by the European Medicines Agency, the Food and Drug Administration or Japanese Pharmaceuticals and Medical Devices Agency; therefore, no clinical trials have been carried out to date.

Although many promising results have been achieved, further research needs to be carried out before PHB and PHB composites can be commercialized for biomedical applications. Challenges for the future are cost reduction of the production and extraction of PHB, optimization of PHB regarding all requirements for bone tissue engineering scaffolds and, finally, *in vivo* studies to confirm the usability of PHB composites for bone tissue engineering.

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